

(SsrA-tagged) hIL-3 are indicated by ~>.

Figure 6. Tagging of native *B. subtilis* proteins. Total intracellular or extracellular proteins produced by cells in the exponential growth phase or stationary phase of *B. subtilis* 168 expressing wild-type SsrA (AA), 168 IssrA^{DD} expressing SsrA^{DD} (DD), or 168 ΔssrA containing no SsrA RNA (-) were analyzed by Western blotting using anti-Bs-SsrAtag antibody.

Figure 7. A native protein and examples of the types of tags encompassed by the instant invention. (A) Depicts the sequence of human interleukin-3; the (native) signal peptide is in bold. (B) Depicts the IL-3 sequence encoded by plasmid pLATIL3. The sequence of AmyL[ss]-interleukin-3 is for hIL-3 secretion in *Bacillus*: the AmyL signal peptide is in bold, and this expressed IL-3 lacks the last four amino acids (LAIF) of native hIL-3. The tag is in *italics*. (C) Depicts IL-3 with a tag that is a substitution of the native protein's terminal two amino acids. The tag is in *italics*. (D) Depicts a tag that is an addition to the native protein's carboxy terminus. Here the sequence of hIL3 as encoded by pLATIL3 with the SsrA-DD tag at the C-terminus [hIL3-DD] (signal peptide in **bold**, C-terminal tag in *italics*) is shown.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only using the following definitions and examples. All patents and publications, including all sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference.

The present invention provides for a process to enhance the production of a desired secreted polypeptide by a suitable host cell. In particular, the present invention may be applicable to increase protein production by *B. subtilis*. Changing the last two C-terminal amino acids residues into at least one, preferably two, charged amino acid residues or adding at least one, preferably two, charged amino acid residues to the COOH-terminus of a protein may be used to increase the yield of any protein

secreted by *B. subtilis*. A longer tag sequence may be utilized in the present invention. Especially the secretion of proteins that have a pI value > 7 may be improved by this concept. In general, the minor alteration (adding or replacing two amino acid residues) itself should not lead to a dramatic change
5 in e.g. the specific activity of an enzyme or the thermostability.

Definitions

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, et al., DICTIONARY OF
10 MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this invention. Although any methods and materials similar or equivalent to those described herein can be used in
15 the practice or testing of the present invention, the preferred methods and materials are described. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. The headings provided herein are
20 not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

Host cell

25 "Host cell" means a cell that has the capacity to act as a host and expression vehicle for an expression cassette according to the invention. In one embodiment, the host cell is a microorganism. In a preferred embodiment according to the present invention, "host cell" means the cells of *Bacillus*. As used herein, the genus *Bacillus* includes all members known to
30 those of skill in the art, including but not limited to *B. subtilis*, *B. licheniformis*,

B. latus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. coagulans, B. cibulans, B. laetus and B. thuringiensis.
Other cells useful in the present invention include *Acinetobacter*, *Thermus*, *Deinococcus Radiodurans*.

5 **Polypeptide or protein**

The term "polypeptide" as used herein refers to a compound made up of amino acid residues linked by peptide bonds. The term "protein" as used herein may be synonymous with the term "polypeptide" or may refer, in addition, to a complex of two or more polypeptides.

10 Additionally, a "protein of interest" or "polypeptide of interest" refers to the protein to be expressed and secreted by the host cell. The protein of interest may be any protein that up until now has been considered for expression in prokaryotes. The protein of interest may be either homologous or heterologous to the host.

15 The term "chimeric polypeptide" and "fusion polypeptide" are used interchangeably herein and refer to a protein that comprises at least two separate and distinct regions that may or may not originate from the same protein. For example, a signal peptide linked to the protein of interest wherein the signal peptide is not normally associated with the protein of interest would 20 be termed a chimeric polypeptide or chimeric protein.

Signal Sequence

A "signal peptide" as used herein refers to an amino-terminal extension on a protein to be secreted. "Signal sequence" is used interchangeably herein. Nearly all secreted proteins use an amino-terminal protein extension 25 which plays a crucial role in the targeting to and translocation of precursor proteins across the membrane and which is proteolytically removed by a signal peptidase during or immediately following membrane transfer.

In preferred embodiments the signal sequence is selected from sec-dependent signal peptides or tat-dependent signal peptides derived from 30 *Bacillus*.